

09/325,189

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Term:

13 and (low concentration near5 primer\$1 near5
 labeled)

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L2: Entry 7 of 8

File: USPT

DOCUMENT-IDENTIFIER: US 5567583 A

TITLE: Methods for reducing non-specific priming in DNA detection

Brief Summary Text (2):

Recent development of the polymerase chain reaction ("PCR") has provided an important tool for the detection of nucleic acid sequences present at low concentrations (Mullis, K. B. et al., U.S. Pat. Nos. 4,683,195 and 4,683,202). In PCR, a target sequence having boundaries defined by two oligonucleotide extension primers, is amplified through repeated enzymatic cycles to provide additional templates for further amplification reactions. Accordingly, a small number of target sequences can be exponentially amplified and readily detected.

Brief Summary Text (3):

A major limitation of PCR lies in the extensive generation of by-products produced as a result of non-specific priming events, e.g., random priming of the nucleic acid template and/or self priming of the extension primers. Thus, when a high number of amplification cycles are required to amplify a target sequence present at a relatively low concentration, the products of non-specific priming events significantly impede PCR sensitivity.

Detailed Description Text (18):

Unlike a universal detection duplex, a "specific detection duplex" is ready for use in the amplification/detection of a target nucleic acid. See FIG. 1. The solid circle and solid square stand for two fluorophores and the hollow circle stands for an incapacitated 3' end of the blocking oligonucleotide. Note that in the embodiment shown in FIG. 1, the labeled primer contains a segment noncontiguous to its priming sequence. Further, a pre-asymmetric amplification, which preferentially amplifies the strand to which the labeled primer binds, is conducted to enhance both the sensitivity and selectivity. An asymmetric reaction can be induced by providing unequal amounts of the primers, i.e., an excessive one and a limiting one, or by providing primers of unequal lengths in complementarity to the specific priming sequence. Other factors may also contribute to the asymmetric nature of amplification, such as the difference in rate for the synthesis of each template strand. The optimal primer ratio for asymmetric amplification is a factor to be determined empirically. See Shyamala et al., 1989, J. Bacteriol. 171:1602, hereby incorporated by reference.